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### CYTOLOGICAL OBSERVATIONS ON TWO TROPICAL FORMS OF *TRIPSACUM*

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SINCE the successful crosses between *Tripsacum dactyloides* L. and maize were reported by Mangelsdorf and Reeves in 1931, the genus *Tripsacum* has become more interesting to both taxonomists and geneticists. Despite this, only a limited number of species in this genus have been cytologically investigated (Longley, 1924 and 1937; Reeves and Mangelsdorf, 1935; Mangelsdorf and Reeves, 1939; Anderson, 1944; Graner and Addison, 1944; Dodds and Simmonds, 1946; Hernández and Randolph, 1950; Prywer, 1954; Maguire, 1957 and 1960). Most of the previous studies consisted of the determination of chromosome numbers and an interpretation of the homology between the chromosomes of *Tripsacum* and those of *Zea*. In addition, some discussed the phylogenetic relationship among different species in the genus (Mangelsdorf and Reeves, Anderson, and Graner and Addison).

The chromosome number of *Tripsacum laxum* Nash has been reported as about  $2n=70$  by Longley (1924) from a study of meiotic divisions. However, based on the mitotic divisions in the root tips, Mangelsdorf and Reeves (1939) stated that the chromosome number was 72.

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Dodds and Simmonds (1946) described the meiosis in this species, and they also found that their material had 72 chromosomes in the root tips. They observed an average of 25–30 bivalents and 10–12 univalents and about two multivalents at diakinesis. They also observed non-congressed univalents and bivalents at metaphase I, laggards at anaphase I, and micronuclei at quartet stage. Finally, they suggested that *Tripsacum laxum* is an amphidiploid, and correlated the sterility of their plants with meiotic irregularity.

Cutler (1947) stated that *Tripsacum laxum*, as well as *T. pilosum* and *T. latifolium*, was usually sterile, and it did not produce any viable seeds.

As the result of a meiotic study on the Brazilian species *Tripsacum australe*, Graner and Addison (1944) concluded that it was a diploid. No knobs were observed on any of the chromosomes, although knobs frequently occur on the chromosomes in other species of the genus.

The present study consists of observations on certain meiotic features in two tropical *Tripsacums* from Colombia, probably referable to *Tripsacum laxum* Nash and *T. australe* Cutler and Anderson, respectively. It is hoped that it will provide some cytological bases for interpreting the phylogenetic relationships of the species concerned.

#### MATERIALS AND METHODS

The clone of *Tripsacum laxum* was first collected near Buenaventura, Colombia, where it had been introduced from Puerto Rico. It is cultivated under the name *caña antigua*. The plants are unusually large and vigorous, even for *T. laxum*, with numerous stout tillers, but rarely set any viable seeds. The staminate spikelets are paired, with one sessile and one pedicellate spikelet. Presumably of hybrid origin, it seemed worthy of cytological investigation.

*Tripsacum australe*, the only native South American species of the genus, is found from Colombia to Bolivia, Paraguay and Brazil. It is very variable in habit. Little is known about its cytology. Graner and Anderson (1944) investigated a large robust clone from Mato Grosso, Brazil, which had eighteen pairs of chromosomes and differed from all other species of *Tripsacum* in lacking terminal knobs on the chromosomes. Since further information regarding this species seemed desirable, a study was made of Colombian plants. These are smaller, with sessile paired staminate spikelets and they usually set fertile seeds.

Material of both these clones was collected for this study by W. H. Hatheway at the Estacion Experimental "Tulio Ospina" at Medellín during the summer of 1956. Inflorescences of the selected clones were fixed in the field in a freshly prepared mixture of three parts of 95% ethyl alcohol and one part of glacial acetic acid. Aceto-carmin squash technique was followed throughout the study.

#### OBSERVATIONS

##### *Tripsacum laxum* Nash

All of the stages of meiotic division, starting at pachytene, were found. Considerable difficulty was encountered in the identification of the chromosomes, because of the extremely irregular chromosome behavior at both pachytene and diakinesis. However, after a number of cells had been studied, it was concluded that this form of *Tripsacum* has 54 chromosomes and is a triploid. This conclusion was finally confirmed by chromosome counts of the tapetal cells in the anthers.

Not a single microsporocyte showed clearly all the chromosomes of the three chromosome sets. Through a number of separate measurements of different cells at



pachytene, the 18 chromosomes of a haploid set could be recognized. The length, arm ratio and spindle fiber attachment region of each chromosome were identified. There are probably three chromosomes (3, 5 and 8) having internal knobs on the long arm. The internal knob on the long arm of chromosome 3 is small and definitely heterozygous. The other two internal knobs are large and homozygous (Plate XX, figs. 1 and 2). Probably only chromosome 9 is knobless. The other 17 chromosomes have one or two knobs terminating one or both arms. However, there is a clear tendency to have the knobs terminating the long arms. Chromosomes 2, 3, 5, 14 and 17 are heterozygous for knobs and prominent chromomeres. In agreement with Longley's report (1937) on *Tripsacum floridanum*, chromosome 16 has a nucleolar organizer on its long arm. This condition is different from that in maize. Furthermore, the secondary constriction of the nucleolar chromosome in *T. laxum* is not always well marked.

Univalent chromosomes were frequently entangled with the bivalent chromosomes in a densely crowded mass. Whenever they were isolated, they demonstrated the spindle fiber attachment region and were readily identifiable. It was also observed that the univalent chromosomes sometimes formed a non-homologous association. Chromosome fragments and loops in the bivalent chromosomes were frequently found at pachytene. Fusions of the chromosome knobs and those of the spindle fiber attachment regions were constantly present.

At diakinesis, eighteen bivalent chromosomes were almost never found; the number of the univalent chromosomes was always greater than eighteen. Multivalent chromosomes were also seen, but only with a low frequency. At metaphase I, many chromosomes often failed to congress in the equatorial plate; the number of lag-

ging chromosomes found varied from eight to seventeen. Laggards at anaphase I were found in every sporocyte. As soon as the division process reached the quartet stage, several micronuclei were always produced around each quartet.

In every preparation, the microsporocytes in this form of *Tripsacum laxum* appeared much larger than those in the form of *T. australe* discussed below.

### *Tripsacum australe* Cutler and Anderson

The pachytene chromosomes in the plants of this species were much easier to study. The number of bivalent chromosomes is definitely 18; in other words, it is a diploid with 36 somatic chromosomes. At late pachytene, spindle fiber attachment regions offered even better working material than those of maize chromosomes. Length and arm ratio of each chromosome could be averaged out as soon as several measurements had been made. Chromosome 8 has a small internal knob on the long arm (Plate XX, fig. 3). As illustrated in Plate XXI, chromosomes 1, 4, 11 and 15 have a knob terminating the long arm. The spindle fiber attachment regions of chromosomes 2, 10 and 16 appeared median. Knobs are not present on the short arms of any of the eighteen chromosomes.

As shown in Plate XXI, it is possible to divide the chromosomes into two groups, A and B; group A has the nine long chromosomes, and group B the nine short ones. The length of the shortest chromosome among the nine long chromosomes in group A is about equivalent to the length of maize chromosome 10. The length of the longest chromosome among the nine long chromosomes of the same group is about equivalent to the length of maize chromosome 4. Nevertheless, the arm ratios of most of the nine long chromosomes are differ-



ent from maize chromosomes of comparable length.

The pachytene chromosomes of this tropical form of *Tripsacum australe*, and those of the tropical form of *T. laxum*, as well, are, for unknown reasons, more heteropycnotic than those of maize, especially along the short arms of the nine short chromosomes.

Chromosome behavior was regular during all the stages of meiotic division. At diakinesis most of the homologues paired lengthwise, with occasionally one or two bivalents associated end-to-end. Univalents, however, were rarely found.

### DISCUSSION

One of the possibilities that may account for the origin of polyploidy in plants is by interspecific hybridization. The characteristics of the triploid form of *Tripsacum laxum* reported in this paper suggest that it probably originated in this manner. The evidence includes the following observations: (1) the pachytene chromosomes are heterozygous; (2) the chromosome behavior is extremely irregular at meiosis; (3) viable seeds are rarely produced; and (4) more univalents than trivalents are found at diakinesis.

In contrast to a triploid maize which Randolph and McClintock (1926) suggested had originated by the mating of two gametes with the chromosome number of one of them became doubled in premeiotic division, the present triploid form of *Tripsacum laxum* probably had a tetraploid *T. laxum* as one of its ancestors and an unknown diploid as the second parent. According to the observations of the pachytene chromosomes, these hypothetical parental species were, at least, different in chromosomes 2, 3, 5, 14 and 17. As stated in the foregoing section, these chromosomes were consistently found heterozygous for knobs and large chromomeres, and in addition, they often failed to associate regularly.

## PLATE XX

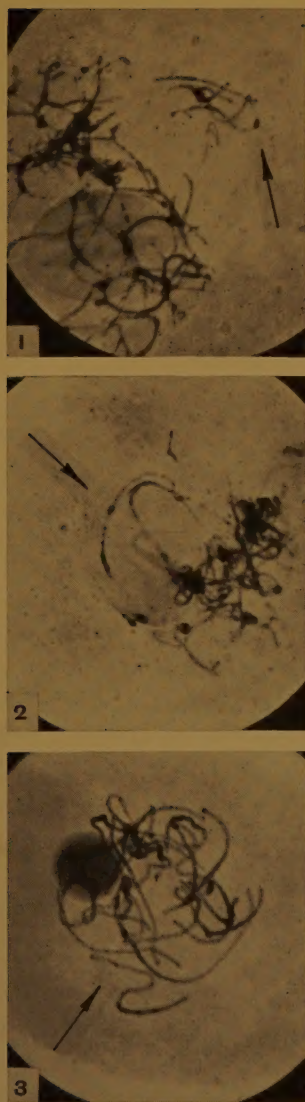


PLATE XX. 1, 2. Photomicrographs of the pachytene chromosomes in the microsporocytes of triploid *Tripsacum laxum* Nash. The arrows indicate the internal knobs on the long arm of chromosome 5 (fig. 1) and on that of chromosome 8 (fig. 2). 750 $\times$ .

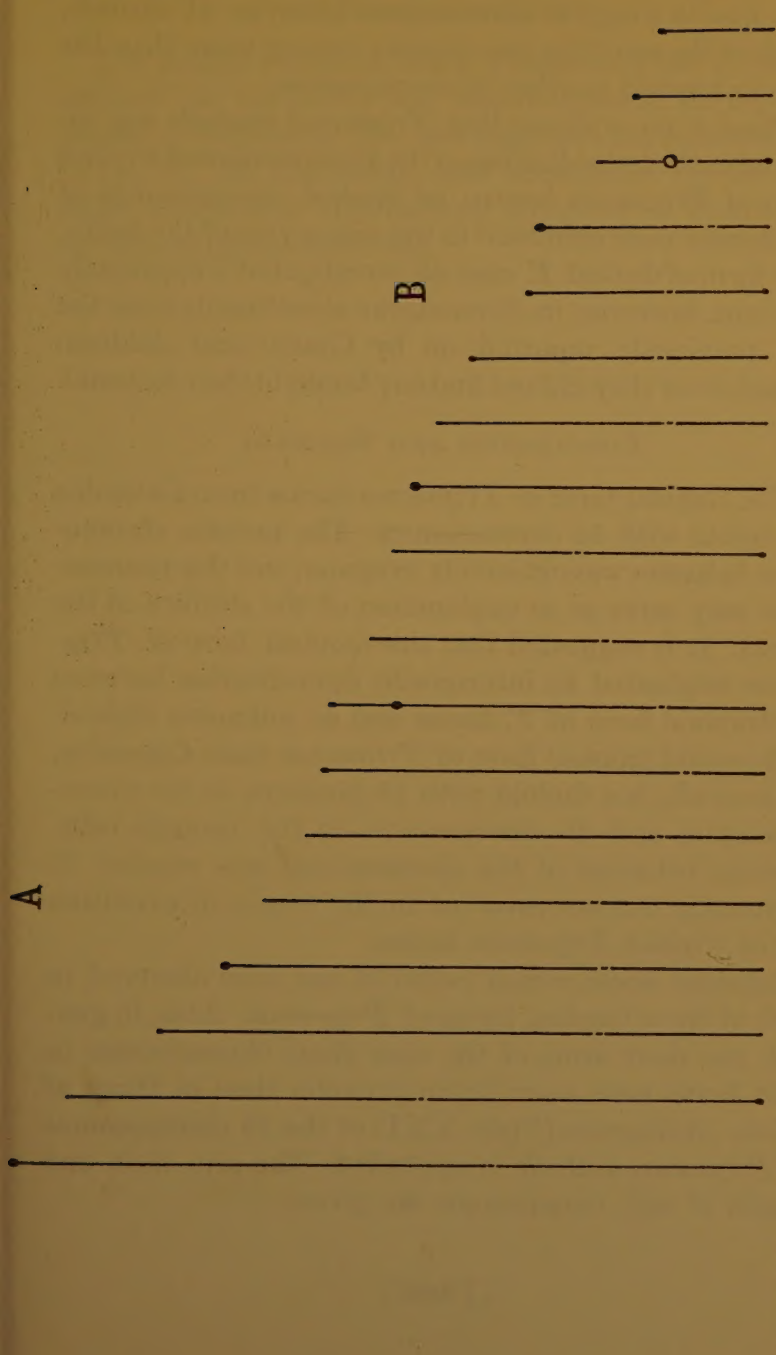
3. Photomicrograph of the pachytene chromosomes in the microsporocyte of *Tripsacum australe* Cutler and Anderson. Arrow points to the internal knob on the long arm of chromosome 8. 750 $\times$ .

## EXPLANATION OF THE ILLUSTRATION

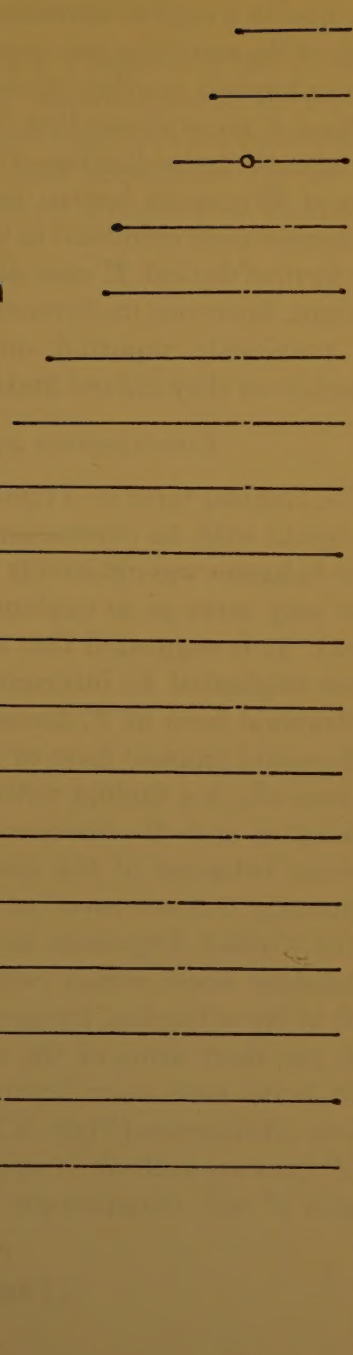
PLATE XXI. Diagram of the 18 chromosomes in *Tripsacum australe*. The lengths, arm ratios, spindle fiber attachment regions (broken line), large chromomeres (small dots), and knobs (large dots) of the chromosomes are determined by actual measurements and observations at pachytene in the microsporocytes. Chromosome 16 has a nucleolar organizer (circle) in the long arm. The chromosomes are divided into two groups, A and B: group A has the nine long chromosomes, group B, the nine short chromosomes.



A



B



Length (u)	68.8	66.0	57.1	52.5	45.3	42.8	42.0	40.4	37.5	34.1	32.6	31.0	26.6	23.1	22.0	16.0	13.2	10.8
Arm ratio (Long/Short)	1.7	1.0	2.8	2.8	2.0	3.4	4.8	3.4	1.4	1.0	2.9	2.5	1.8	1.5	3.3	1.2	2.7	3.8
Chromosome	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18

Sterility of the clone of *Tripsacum laxum* is undoubtedly due to irregular chromosome behavior at meiosis. Most of the resulting microspores receive more than the regular haploid number of chromosomes.

There is no evidence that *Tripsacum australe* was involved in the hybridization of the aforementioned triploid form of *Tripsacum laxum*; no marked chromosomes of the former were identified in the sporocytes of the latter. The form of diploid *T. australe* investigated is apparently different, however, in chromosome constituents from the one previously reported on by Graner and Addison (1944), since they did not find any knobs in their material.

### CONCLUSIONS AND SUMMARY

The tropical form of *Tripsacum laxum* from Colombia is triploid with 54 chromosomes. The meiotic chromosome behavior was extremely irregular, and this phenomenon may serve as an explanation of the sterility of the plants. It is suggested that this tropical form of *Tripsacum* originated by interspecific hybridization between a tetraploid form of *T. laxum* and an unknown diploid.

A second tropical form of *Tripsacum* from Colombia, *T. australe*, is a diploid with 18 bivalents in the microsporocytes and 36 chromosomes in the somatic cells. Meiotic behavior of the chromosomes was regular. It apparently was not involved in the course of evolution of the triploid *Tripsacum laxum*.

Internal knobs which varied in size were observed in both of these tropical forms of *Tripsacum*. Also, in general, the short arms of the nine short chromosomes in both forms were more heteropycnotic than in those of maize. A diagram (Plate XXI) of the 18 chromosomes in *Tripsacum australe* is appended. The arm ratio and length of each chromosome are given.

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